AD_____

GRANT NO:

DAMD17-94-J-4029

TITLE:

New Approaches To The Labeling Of Estrogens Useful For PET

PRINCIPAL INVESTIGATOR:

Stephanie D. Jonson

CONTRACTING ORGANIZATION:

Washington University
St. Louis, Missouri 63110

REPORT DATE:

June 29, 1995

TYPE OF REPORT:

Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release;
distribution unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE: June 29, 1995	3. REPORT TYPE AND DATES COVERED: Annual Report: 6/1/94 - 5/31/95

4. TITLE AND SUBTITLE

New Approaches To The Labeling Of Estrogens Useful For PET

5. FUNDING NUMBERS

DAMD17-94-J-4029

6. AUTHOR(S)

Stephanie D. Jonson

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Washington University
724 South Euclid Avenue
St. Louis, Missouri 63110

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

US Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for Public Release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

Progress: During this year, research has focused on understanding the chemistry of methyl hypofluorite (MeOF). MeOF is the only source of the novel electrophilic methoxylium ion species MeO^+ and has been shown to react readily with C-C double bonds. Quick incorporation of C-11 with the help of MeOF will allow radiolabeling of steroids useful for positron emission tomography (PET) to disclose the location of tumor lesions and metastases with a lower dose of radiation to the patient. An estradiol derivative, 16α-methoxy estradiol, was selected as the target molecule and synthesized. Radiolabeling of the estradiol derivative with the use of [C-11]CH_3OF was attempted, but yields were low. One of the goals for the following year is to increase this yield. Yields are being optimized by replacing the reaction solvent with radical scavenging chloroform. During the next year, the investigator will optimize radiochemical reactions for the target steroidal molecules and probe their binding affinities in tumor sites.

14. SUBJECT TERMS

positron tomography, estrogens, carbon-11, receptor ligands, cholesterol

15. NUMBER OF PAGES: 11

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the US Army.

____ Where copyrighted material is quoted, permission has been obtained to use such material.

____ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

____ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

____ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

____ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

____ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

____ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

____ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Accesion For	
NTIS CRA&I	☒
DTIC TAB	☐
Unannounced	☐
Justification	
By	
Distribution /	
Availability Codes	
Dist	Avail and / or Special
A-1	

Stephanie D Jones 6/29/95
PI - Signature Date

Table Of Contents

	Page
A. Introduction	2
B. Body of Report: Experimental Results and Discussion	2
C. Conclusion: Future Plans	5
D. Statement of Work Time Line	6
E. References	7
F. Appendix 1: Abstract of Oral Presentation	8

New Approaches To The Labeling Of Estrogens Useful For PET

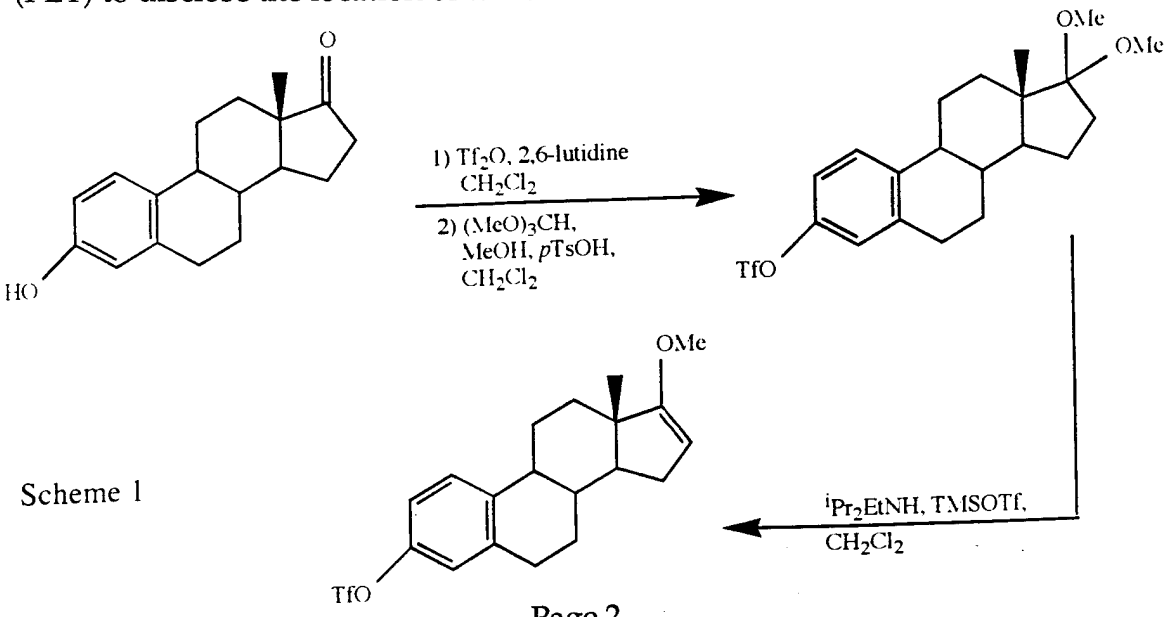
Introduction

The first year of Army funding has coincided with my second year as a graduate student at Washington University in St. Louis, Missouri. Progress toward my doctoral degree in nuclear chemistry this past year includes fulfillment of required courses and laboratory teaching as well as successful completion of all candidacy exams. Passing both the written and oral candidacy exam allows me to continue in the doctoral program in nuclear chemistry. I will obtain a Master's Degree in August 1995 as a result of fulfilling these requirements.

Research goals for the past year focused on understanding the chemistry of methyl hypofluorite (MeOF) and its application to radiolabeling estrogens with C-11. MeOF is the only source of the novel electrophilic methoxylium ion species "MeO^+" and has been shown to react readily with C-C double bonds[1] and various types of methyl enol ethers.[2] Quick incorporation of C-11 with the help of MeOF will allow radiolabeling of steroids useful for PET.

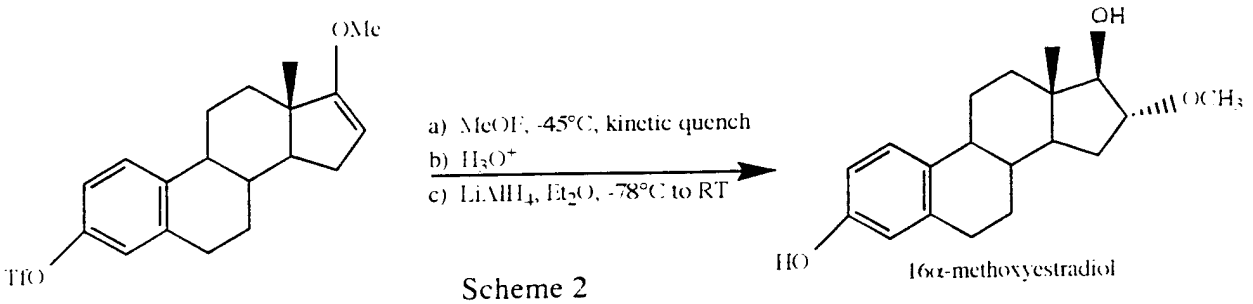
Experimental Results and Discussion

The main objective was to synthesize an estrogen analogue that has a high binding affinity for the estrogen receptor found in estrogen receptor positive breast tumors.[3] C-11 radiolabeling could then be obtained through the use of [C-11]CH_3OF. This would enable the use of positron emission tomography (PET) to disclose the location of tumor lesions and metastases.



Scheme 1

Searching for a biologically interesting target molecule led to the selection of 16α-methoxy estradiol. The initial substrate synthesized in route to the target molecule was the 17-methylenolether of estrone protected at the 3 position with triflate (scheme 1). Upon reaction with methyl hypofluorite and H_3O^+, the 16α-methoxy-3-triflate estrone was obtained. Reduction and deprotection with $LiAlH_4$ produced the estradiol derivative with a yield of ~10% (scheme 2). This estradiol derivative is expected to be less lipophilic than estradiol based on its calculated log P values (2.86 and 4.63 respectively). Lower lipophilicity is advantageous for it reduces non-specific binding. Binding studies will be done to determine the affinity for the estrogen receptor positive target tissue.

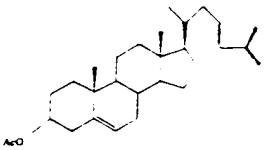


Scheme 2

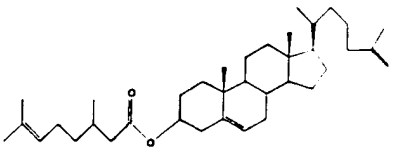
Radiolabeling of the estradiol derivative is accomplished with the use of [C-11]CH_3OF. This reaction is being optimized as the yields are currently low. This work was presented at the National American Chemical Society Meeting in August 1994 and the abstract can be found in Appendix 1.

Desiring to better understand the chemistry of MeOF, a model compound was sought to probe its reactivity. Cholesterol was chosen because it has a rigid steroidal skeleton and internal double bond. Counsell and coworkers have reported radioiodinated esters of cholesterol as potentially useful tumor imaging agents.[4,5,6] Carbon-11 labeled cholesterol esters would induce a lower radiation dose on the patient (due to the shortened half-life) and would allow for more rapid imaging. With this in mind, various ester linkages each containing one double bond were attached to cholesterol at the 3 position. Therefore, each cholesterol analogue contained 2 double bonds allowing an investigation in to which one MeOF would prefer. The objective was to make the external double bond more reactive than the internal double bond. This would allow the molecule to be radiolabeled on the ester linkage with the help of [C-11]CH_3OF.

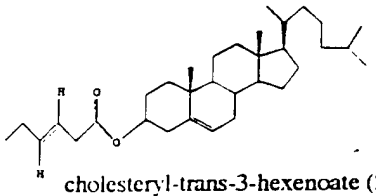
Rozen and coworkers have probed the reactivity of methyl hypofluorite with olefins.[1] The more electron-rich double bonds were found to react preferentially with MeOF. Cholesterol acetate (**1**) which contains an internal tertiary double bond reacted with MeOF to produce 5α-fluoro-6β-methoxy cholestanol acetate with a yield of 20%. Desiring to probe the reactivity of the external double bond in the presence of the internal double bond, various cholesterol esters containing a secondary double bond (**2**), tertiary double bond (**3**), terminal tertiary double bond (**4**), and a benzylic double bond (**5**) were synthesized and reacted with MeOF.



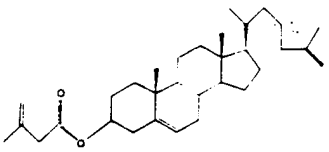
cholesteryl acetate (1)



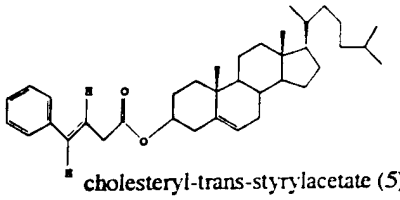
cholesteryl citronellate (3)



cholesteryl-trans-3-hexenoate (2)



cholesteryl-3-methyl-3-butenoate (4)



cholesteryl-trans-styrylacetate (5)

Reacting cholesteryl-trans-3-hexenoate (**2**) with MeOF only resulted in addition of MeOF to the internal double bond as identified by NMR. This was as expected for the internal tertiary double bond is more electron rich than the external secondary double bond. The tertiary internal double bond of cholesterol had provided a more stable cation intermediate which has been shown to occur during the addition of MeOF.[2] A solubility problem was noticed during the reaction of MeOF and the steroid. Methyl hypofluorite is generated in acetonitrile at -45°C, but the steroidal substrates are not soluble in acetonitrile. The solubility problem has been solved by adding the methyl hypofluorite/acetonitrile to the substrate dissolved in methylene chloride providing a 2:1 solution of acetonitrile and methylene chloride. The cholesteryl-citronellate (**3**) molecule exposed MeOF to two tertiary double bonds, however, the internal double bond provided a less hindered environment. No external addition of MeOF was observed.

The rigidity of the steroidal skeleton provides an unhindered attack path for MeOF. It was desired to make the external double bond less hindered to increase its reactivity. The chosen molecule was cholesteryl-3-methyl-3-butenoate (**4**) which possesses a terminal tertiary double bond. When reacted with MeOF, NMR showed the that the internal double bond was preferred to the external double bond by a ratio of 4:3. The product containing the internal double bond was isolated from the reaction mixture and characterized by ^{1}H NMR and high resolution mass spectrometry.

With the benzylic external double bond (**5**), addition of MeOF preferred the external double bond to the internal double bond 4:1. The electron rich benzylic double bond proved to be more reactive than the others. The benzylic compound is also advantageous for it is UV active. This allows the reaction to be purified and monitored by HPLC using a UV detector at λ=254 nm. HPLC conditions are currently being determined for the reaction of cholesteryl-trans-styrylacetate with methyl hypofluorite. Soon to follow will be the reaction with [C-11]CH_3OF to probe the preference for the internal double bond to the external benzylic double bond at the tracer level.

Conclusion: Future Plans

This past year has provided valuable experience for working with methyl hypofluorite. Unseen problems arose when the chemistry of methyl hypofluorite was combined with steroids. Difficulties with solubility have been solved and the obstacle of purification continues to be rectified. Yields are being optimized by replacing the reaction solvent, currently methylene chloride, with radical scavenging chloroform. These problems have been counteracted as they have arisen providing the development of problem solving strategies needed in the research field. I look forward to optimizing the syntheses of the non-radioactive molecules in the coming year and hopefully to carry out binding studies and radiochemical syntheses. This will be ahead of my original schedule. A time line for the statement of work follows.

Time Line for Statement of Work

Goal	Year of Funding 0	1	2	3	4
1) Complete Required Course Work		---------->			
	..........>complete				
2) Synthesize Authentic Non-Radioactive Compounds		---------->			
	.....				
3) Measure Binding Affinity of Non-Radioactive Compounds			--------->		
4) Synthesize Radioactive Compounds				-------->	
5) Evaluate Best Compounds in Animal Models				---------->	
6) Write Thesis				--->	

----------> represents original time estimate

..........> represents work completed

References

1. Rozen S, Mishani E, Kol M, and Ben-David I, *J. Org. Chem.*, 1994, 59, 4281-4.
2. Rozen S, Mishani E, Kol M, *J. Am. Chem. Soc.*, 1992, 114, 7643-5.
3. Katzenellenbogen J, In *Radiopharmaceuticals: Chemistry and Pharmacology*; A.D. Nunn, Ed.; Marcel Dekker, Inc.: New York, 1992, Vol. 55, 297-331.
4. Seevers RH, Schwendner SW, Swayze SL, Counsell RE, *J. Med. Chem.*, 1982, 25, 618-621.
5. van Dort M, Santay L, Schwendner SW, Counsell RE, *Appl. Radiat. Isotopes*, 1989, 16, 603-7.
6. Counsell RE, Seevers RH, Korn N, Schwendner SW, *J. Med. Chem.*, 1981, 24, 5-6.

Appendix 1

Abstract of Oral Presented at the National American Chemical Society Meeting in Washington, DC, August 1994.

42. CHEMISTRY AND RADIOCHEMISTRY OF NO-CARRIER ADDED $^{11}CH_3OF$
Stephanie D. Jonson, Thomas A. Bonasera, Timothy J. McCarthy, Michael J. Welch, Mallinckrodt Institute of Radiology, Washington University School of Medicine, St. Louis, MO, 63110.

Methyl hypofluorite has been reported to undergo reactions with various enol ethers to furnish α-methoxy ketones. This new synthetic intermediate is the only source of methoxylium ion "MeO$^+$" (S. Rozen, E. Mishani, and M. Kol. *J. Am. Chem. Soc.*, Vol. 114, No. 20, 1992) and has allowed for several novel reactions to be developed. We have developed $^{11}CH_3OF$ and synthesized model α-methoxy ketones. The use of this radioactive reagent allows for the introduction of carbon-11 into various substrates. Methyl hypofluorite is being utilized to produce radioactive and non-radioactive novel steroids. The approaches to the design of these novel steroidal structures will be described.